

**CLAIMS:**

1. A method for producing a highly pure population of polyclonal Th1 memory cells, comprising:
  - collecting source material from a subject;
  - purifying T-cells from the source material; and
  - activating the T-cells a minimum of 3 times at 2-4 day intervals, whereby a highly pure population of polyclonal Th1 memory cells is produced.
2. The method of claim 1, wherein the T-cells are purified CD4+ cells.
3. The method of claim 2, wherein the CD4+ cells are purified by positive selection
4. The method of claim 3 wherein the CD4+ cells are purged of CD45RO+ cells
5. The method of claim 1, wherein the source material is purged of platelets
6. The method of claim 4, wherein the source material is purged of platelets
7. The method of claim 1, wherein the source material is purged of monocytes.
8. The method of claim 6, wherein the source material is purged of monocytes.
9. The method of claim 1, wherein the activation of T-cells is effected by contacting the cells with immobilized anti-CD3 and anti-CD28 mAbs.
10. The method of claim 9, wherein the anti-CD3 and anti-CD28 mAbs are immobilized.
11. The method of claim 10, wherein the beads are initially administered to the purified T-cells at a 3:1 bead:cell ratio and subsequently at a 1:1 bead:cell ratio.

12. A method comprising:

(a) collecting a sample of mononuclear cells from a subject with a disease characterized by either an excess of Th2 cytokine activity or low Th1 cytokine activity; and

5 (b) processing the mononuclear cells *ex vivo* without the use of any exogenous cytokines to produce an expanded population of highly pure Th1 memory cells.

13. The method of claim 12, further comprising:

10 (c) infusing the Th1 memory cells into a subject, thereby altering the Th1/Th2 cell balance of the subject.

14. The method of claim 13, wherein the subject is the donor.

15. The method of claim 13, wherein the expanded population comprises at least  $10^9$  Th1 cells.

16. The method of claim 15, wherein the  $10^9$  cells are in a  
15 volume of about 1 liter or less.

17. The method of claim 12, wherein the disease is selected from the group consisting of diseases characterized by suppression of the cellular immune response or by over-expression of the humoral immune response.

20 18. The method of claim 12, wherein the disease is selected from the group consisting of cancer, infectious diseases, autoimmune and allergic diseases.

19. The method of claim 12, wherein processing is effected by a method, comprising: purifying CD3+ cells from the mononuclear cells.

25 20. The method of claim 12, wherein processing is effected by a method, comprising purifying CD3+ CD4+ cells from the mononuclear cells.

30 21. The method of claim 12, wherein processing is effected by a method, comprising purifying CD3+, CD4+, CD45RA+ cells from the mononuclear cells.

22. The method of claim 12, wherein processing is effected by a method, comprising:

- (i) reducing the platelet concentration in the sample;
- (ii) purging the CD45RO + cells from the population of mononuclear cells;
- (iii) purifying by positive selection a population of CD4 + , CD45RA + cells;
- (iv) activating the resulting CD4 + cells in the absence of exogenous cytokines with immobilized anti-CD3/anti-CD28 mAb;
- (v) periodically restimulating with immobilized anti-CD3/anti-CD28 mAb.

23. The method of claim 22, wherein the cells are restimulated every 2-3 days with immobilized anti-CD3/anti-CD28 mAb for a total of 10-14 days.

24. The method of claim 23, further comprising:

(c) infusing the Th1 memory cells into a subject, thereby altering the Th1/Th2 cell balance of the subject.

25. The method of claim 24, wherein the subject is the donor.

26. The method of claim 24, wherein the expanded population comprises at least  $10^9$  Th1 cells.

27. The method of claim 26, wherein the  $10^9$  cells are in a volume of about 1 liter or less.

28. The method of claim 12, wherein processing is effected by a method, comprising:

- (i) reducing the number of platelets in the sample;
- (ii) purging macrophages from the sample;
- (iii) purging the CD45RO + cells from the sample
- (iv) purifying by positive selection a population of CD4 + , CD45RA + cells;
- (v) activating the CD4 + cells in the absence of exogenous cytokines with immobilized anti-CD3/anti-CD28 mAb; and

(vi) periodically restimulating with immobilized anti-CD3/anti-CD28 mAb.

29. The method of claim 28, wherein the cells are restimulated every 2-3 days with immobilized anti-CD3/anti-CD28 mAb for a total of  
5 10-14 days.

30. The method of claim 28, further comprising:

(c) infusing the Th1 memory cells into a subject, thereby altering the Th1/Th2 cell balance of the subject.

31. The method of claim 28, wherein the subject is the donor.

10 32. The method of claim 28, wherein the expanded population comprises at least  $10^9$  Th1 cells.

33. The method of claim 30, wherein the  $10^9$  cells are in a volume of about 1 liter or less.

34. A composition comprising at least 70% polyclonal memory  
15 Th1 cells.

35. The composition of claim 34, comprising at least  $10^9$  Th1 memory cells.

36. The composition of claim 35 that has density of cells greater than about  $10^6$  cells per ml or  $10^7$  cells per mol or  $10^8$  cells per ml.

20 37. The method of claim 1, wherein the polyclonal Th1 memory cells are activated.

38. The composition of claim 34, wherein the Th1 cells are CD3+, CD4+, CD45RO+, CD62L-, CD44+ and CD25+.

39. A method of treating a disease, comprising:  
25 infusing a composition of claim 34 into a subject with symptoms of a disease, wherein:

the disease is characterized by suppression of the cellular immune response, by over-expression of the humoral immune response, excess Th2 activity or a lack or decreased Th1 activity.

40. The method of claim 39, wherein the disease is selected from the group consisting of cancer, infectious diseases and allergic diseases.

41. A process for producing compositions comprising at least  
5 70% Th1 cells, comprising:

(a) collecting a sample of mononuclear cells from a subject with a disease characterized by either an excess of Th2 cytokine activity or lack of Th1 cytokine activity;

(b) removing platelets from the sample;

10 (c) removing macrophages from the sample;

(c) depleting CD45RO+ cells from the sample by negative selection;

(d) selecting the CD4+ cells by positive selection; and

(e) expanding and differentiating the selected CD4+ cells by  
15 repeatedly stimulating the selected CD4+ cells with immobilized anti-CD3/anti-CD28 antibodies.

42. The method of claim 12, wherein the polyclonal Th1 memory cells are activated.

43. The method of claim 12, wherein the disease is selected  
20 from the group consisting of diseases characterized by excess Th2 activity or a lack or decreased Th1 activity.

44. The method of claim 1, wherein the T-cells are activated 3 to 5 times at 2-4 day intervals.

45. The method of claim 1, wherein the source material  
25 comprises mononuclear cells.

46. The method of claim 1, wherein the subject is a human cancer patient.

47. A process for producing compositions have an enhanced population of activated polyclonal Th1 memory cells, comprising:

30 (a) collecting a sample of mononuclear cells from a subject;

(b) expanding and differentiating the mononuclear cells by repeatedly activating T-cells in the mononuclear cell sample in the absence of exogenous growth or differentiation factors, thereby producing a highly pure population of activated polyclonal Th1 memory cells.

48. The method of claim 47, wherein prior to expanding and differentiating the T-cells are purified from the mononuclear cells.

49. The method of Claim 48 where the T-cells purified from the mononuclear cells are selected from the group consisting of CD3+ cells, CD4+ cells, CD4+, CD45RA+ cells and CD4+, CD45RO+ cells.

50. A method for expanding T-cells from cancer patients without the use of exogenous cytokines, comprising:

- (a) collecting a mononuclear cell sample from a cancer patient;
- (b) purging platelets from the mononuclear cells; and
- (c) activating the cells with immobilized anti-CD3/anti-CD28 mAbs, wherein all steps are performed in the absence of exogenous cytokines.

51. A composition of cells, comprising at least about  $10^9$  cells, wherein at least about 70% of the cells are polyclonal Th1 memory cells.

52. The composition of claim 51, wherein the Th1 cells are activated.

53. The composition of claim 51 that is in a volume of about a liter or less.

54. A composition of polyclonal Th1 memory cells produced by the method of claim 1.

55. A composition of activated polyclonal Th1 memory cells produced by the method of claim 47.

56. A combination, comprising a composition of claim 34 and an immunizing antigen.

57. The method of claim 18, wherein the disease is cancer.

58. The method of claim 57, wherein the disease is selected from the group consisting of liver, kidney, breast, prostate, melanoma, colon, lymphoma, lung, pancreatic, ovarian, esophageal, head and neck, brain, uterine and stomach cancer.

- 5        59. The method of claim 12, wherein the disease is selected from the group consisting of liver, kidney, breast, prostate, melanoma, colon, lymphoma, lung, pancreatic, ovarian, esophageal, head and neck, brain, uterine and stomach cancer.

- 10       60. The method of claim 39, wherein the disease is selected from the group consisting of liver, kidney, breast, prostate, melanoma, colon, lymphoma, lung, pancreatic, ovarian, esophageal, head and neck, brain, uterine and stomach cancer.

61. The method of claim 9, wherein the anti-CD3 and anti-CD28 mAbs are immobilized on immunomagnetic beads.

- 15       62. The method of claim 12, wherein:  
         at least  $10^{10}$  cells are produced; and  
         at least 70% are positive for internal interferon- $\gamma$ .

63. The method of claim 62, wherein the cells are in a volume of about 1 liter or less.

- 20       64. The method of claim 62, wherein the density of cells is at least  $10^{11}$  cells/liter.

65. A high pure population of Th1 cells, at least 70% are positive for internal interferon- $\gamma$  and the cells are at a density of at least  $10^{10}$  cells/liter.

- 25       66. The cells of claim 65, wherein the density of cells is at least  $10^{11}$  cells/liter.